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Basidiospore ultrastructure of some *Dacrymycetales* from Mexico

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Abstract — An ultrastructural study was made of basidiospores from nine species of *Dacrymycetales*: *Calocera macrospora*, *C. viscosa*, *Dacrymyces dictyosporus*, *D. chrysospermus*, *Dacryopinax elegans*, *Da. spathularia*, *Da. lowyi*, *Guepiniopsis alpina,* and *G. buccina*. Spore walls and septa analyzed with transmission electron microscopy revealed the absence or presence of discrete lateral wall layers as well as septal pores that appear diagnostic at the species level.

Key words —jelly fungi, morphology, systematics, taxonomic novelty

Introduction

Septum ultrastructure has been shown to serve as a highly significant phylogenetic marker of the order *Dacrymycetales* (Grand & Moore 1971, Khan & Talbot 1976, Moore 1978, 1994; Khan & Kimbrough 1982, Berbee & Wells 1988, 1989; Boehm & McLaughlin 1989, Lu & McLaughlin 1991, Wells 1994). Even when teleomorphic characters are lacking, the septal pore ultrastructure together with some other character (e.g. pigmentation) facilitates placement of certain taxa within the order (Kirschner & Yang 2005). It is, however, not clear whether there are other ultrastructural characters that permit differentiation at the specific, generic, or family level within the order. Here we describe and compare the ultrastructure of basidiospore walls and septa in several taxa of *Dacrymycetales* from Mexico.

Materials & methods

Seventy-five specimens representing nine dacrymycete species from México were collected. Species represented were *Calocera macrospora, C. viscosa*, *Dacrymyces*

chrysospermus, *D. dictyosporus, Dacryopinax elegans, Da. lowyi, Da. spathularia, Guepiniopsis alpina,* and *G. buccina.* The specimens were examined using standard mycological techniques (Martin 1952, Lowy 1971, Cifuentes et al. 1986, Sierra 1992, 1995, 2000; Sierra & Cifuentes 1993) and deposited in FCME and TLXM herbaria (Holmgren & Holmgren 1990).

Ten specimens with a large number of spores were selected for processing with transmission electron microscopy (TEM). Small (2 mm^3) basidiome pieces were rehydrated for 1 day in distilled water. Samples were fixed with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4 for 2 hours. A post-fixation with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer was carried out for 2 hours. The samples were dehydrated via a graded ethanol series (70–100%), prepared with a mixture of propylene oxide and epoxy resin (in 2:1, 1:1, and 1:2 proportions) for 24 hours, and embedded in pure epoxy resin. The block face was then shaped to a 0.1 mm^2 trapezoid. Sections were cut with glass cleavers in a Sorvall Ultramicrotome MT2. No serial-sections were made due to the scarcity of herbarium materials in good condition. Sections were placed on copper grids and stained in uranyl acetate (10 minutes) followed by lead citrate (5 minutes). The grids were examined with a Zeiss EM-10 TEM.

Species & collections

Calocera macrospora Brasf., Lloydia 1: 156. 1938. Figs 1a,b

Specimen examined: **MEXICO, Tlaxcala**, Mpio. de Tlaxco, Parque recreativo El Rodeo, 19°39′54″N 98°08′17″W, alt. 2900 m, on rotten wood, 14 VIII 1992, Pérez-Ramírez 1604 (FCME 4856).

Calocera viscosa (Pers.) Fr., Syst. Mycol. 1: 486. 1821. Figs 1c,d

Specimen examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Desviación al Balneario Erendira, km 5.5 carr. San Pedro Jacuaro–Los Azufres, 19°45′36″N 100°41′21″W, alt. 2600 m, on soil, 4 IX 1987, Ojeda y Villegas-Ríos 952 (FCME 14002).

Dacrymyces chrysospermus Berk. & M.A. Curtis, Grevillea 2: 20. 1873. Figs 3a,b

Specimen examined: **MEXICO, Guerrero**, Mpio. de Chilpancingo de los Bravo, Cañada de Agua Fría, 17°32′30″N 99°41′30″W, alt. 2500 m, on rotten wood, 8 VII 1984, López L. s/n (FCME 13012).

Dacrymyces dictyosporus G.W. Martin, Mycologia 50: 939. 1959. Figs. 2

Specimen examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Desviación al Balneario Erendira, km 5.5 carr. San Pedro Jacuaro-Los Azufres, 19°45′36″N 100°41′21″W, alt. 2600 m, on rotten wood, 4 IX 1987, Molina y Pérez-Ramírez 805 (FCME 14137).

Dacryopinax elegans (Berk. & M.A. Curtis) G.W. Martin, Lloydia 11: 116. 1948. Fig. 3c

Specimen examined: **MEXICO, Tabasco**, Mpio. de Teapa, Centro Regional Universitario del Sureste (CRUSE) Puyacatengo, 17°31′39″N 92°55′51″W, alt. 80 m, on rotten wood, 20 X 1991, Sierra 46 (FCME 4523).

Dacryopinax lowyi S. Sierra & Cifuentes, Mycotaxon 92: 244. 2005. Figs 3f,g

Specimens examined: **MEXICO, Michoacan**, Mpio. de Zinapecuaro, Laguna Larga, zona de protección forestal Los Azufres, 19°48′05″N 100°41′02″W, alt. 2800 m, on

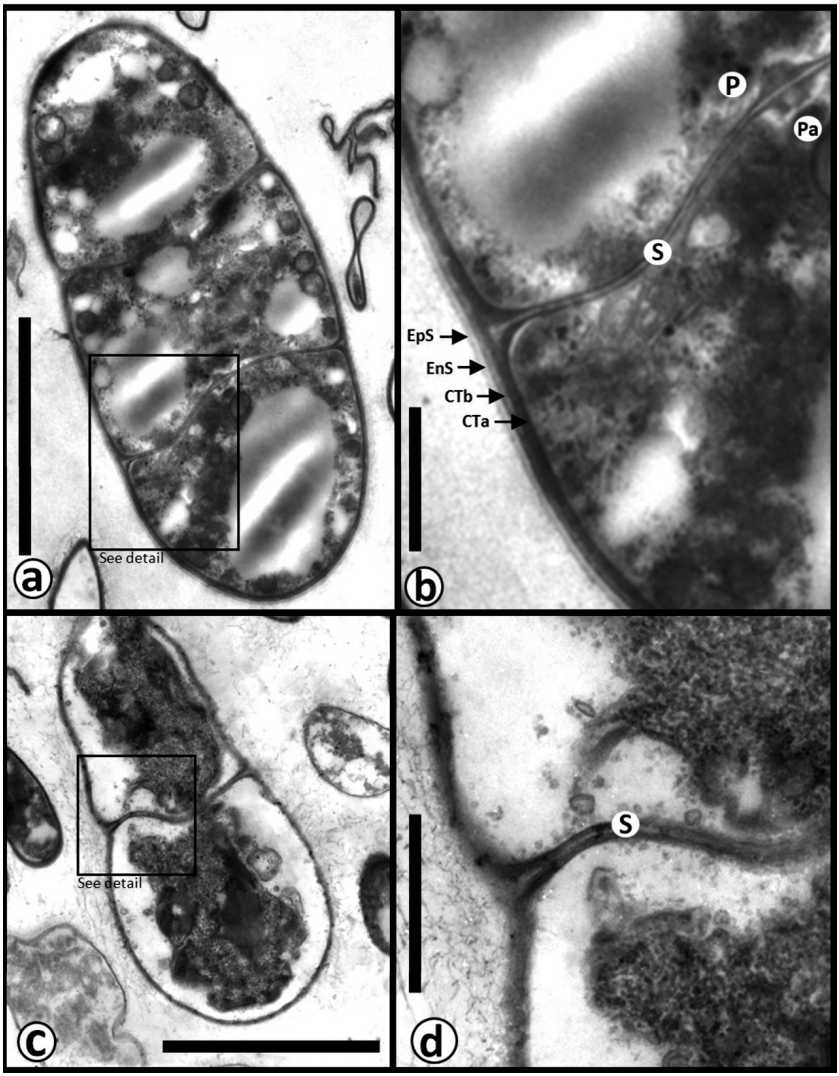

FIGURE 1. ***Calocera macrospora***: **a**. Biseptate basidiospore. **b**. Detail of spore wall and septum [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium= EpS, septum = S, pore = P, pore aperture = Pa]. ***Calocera viscosa***: **c**. Uniseptate basidiospore. **d**. Detail of septal wall [S]. Bars: a, c = 5 μm; b, d = 1 μm.

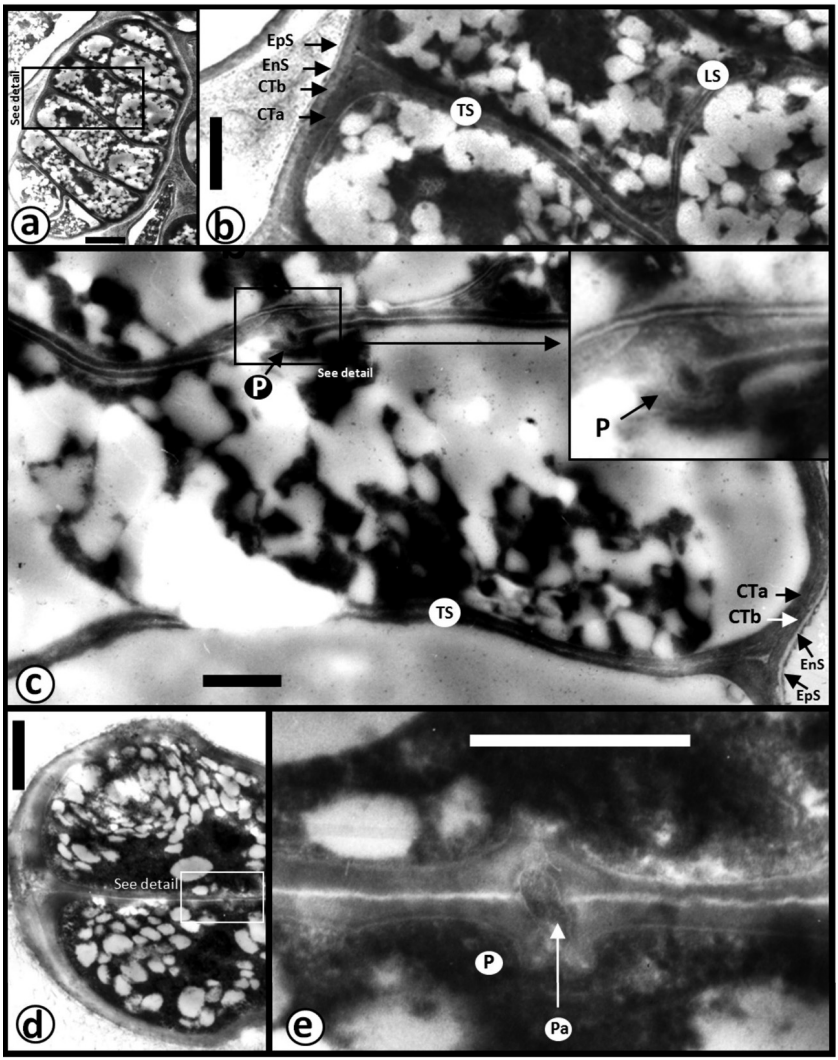

Figure 2. *Dacrymyces dictyosporus*: **a**. Multiseptate basidiospore (transverse and longitudinal septa). **b**. Detail of spore wall and septum [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium = EpS, transverse septum = TS, longitudinal septum = LS]. **c**. Detail of a multiseptate basidiospore; only transverse septum [TS] and spore wall with 4 layers [coriotunic a = CTa, coriotunic b = CTb, endosporium = EnS, episporium = EpS, pore = P]. **d**. Uniseptate basidiospore. **e**. Detail of septal wall [pore = P, pore aperture = Pa]. Bars: a = 5 µm; b–d = 2 µm; e = 1 µm.

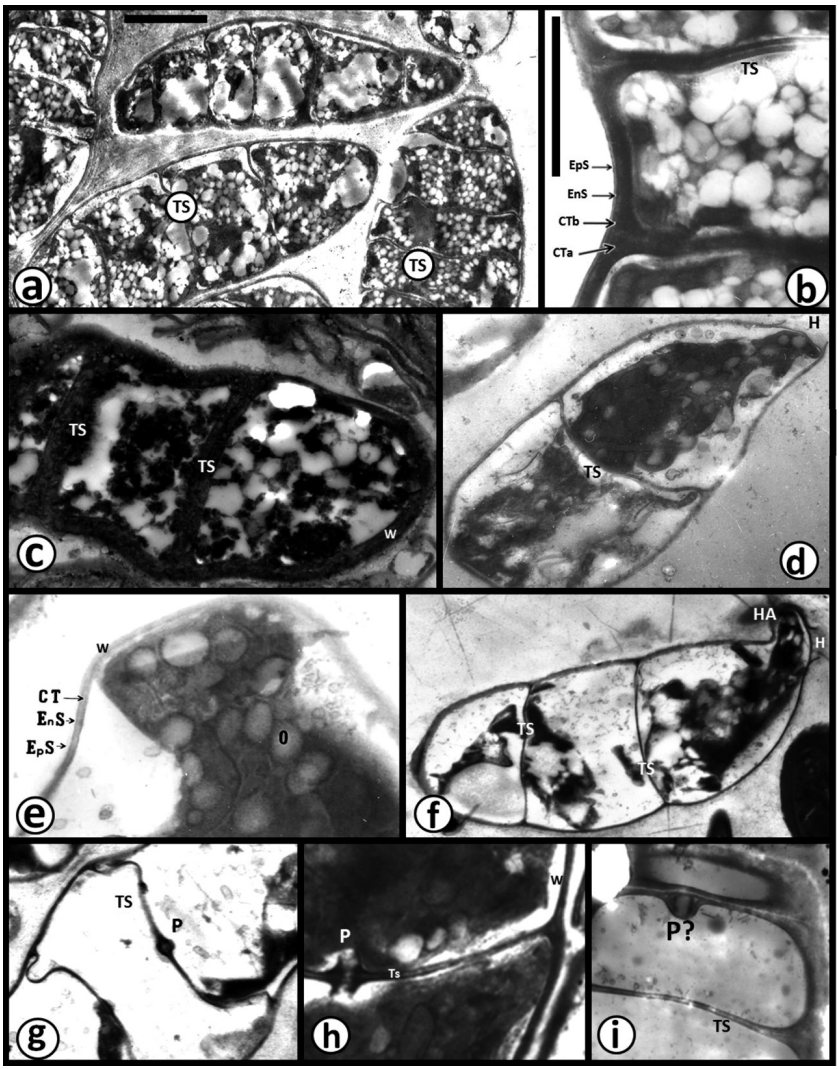

Figure 3. ***Dacrymyces chrysospermus***: **a**. Multiseptate basidiospore with transverse septa [TS] only. **b**. Detail of spore wall and septa. ***Dacryopinax elegans***: **c**. Spore walls [W] and transverse septa [TS]. ***Dacryopinax spathularia***: **d**. Uniseptate basidiospore with hilar appendix [HA]. **e**. Detail of spore wall [W]. ***Dacryopinax lowyi***: **f**. Biseptate basidiospore with hilar appendix [HA]. **g**. Detail of transverse septum [TS] and pore [P]. ***Guepiniopsis buccina***: **h**. Detail of spore wall [W], transverse septum [S], organelle, and pore [P]. ***Guepiniopsis alpina***: **i**. Detail of transverse septum [TS] and pore [P?]. Bar: a, b = 5 μm.

rotten wood, 3 VII 1987, Núñez Mariel s/n (FCME 13863); **Estado de México**, Mpio. de Amecameca, Km 14 carr. Amecameca–Tlamacas, 19°05′14″N 98°40′43″W, alt. 3300 m, on rotten wood, 15 VII 1993, Sierra 165 (FCME 20406).

Dacryopinax spathularia (Schwein.) G.W. Martin, Lloydia 11: 116. 1948. Figs 3d,e

Specimen examined: **MEXICO, Nayarit**, Mpio. de Tepic, La Capilla, Reserva Ecológica del Cerro San Juan, 21°28′11″N 105°00′31″W, alt. 1371 m, on rotten wood, 29 VIII 1991, Rodríguez Castañeda y Pérez-Ramírez 1467 (FCME 3903).

Guepiniopsis alpina (Tracy & Earle) Brasf., Amer. Midl. Nat. 20: 225. 1938. Fig. 3i

Specimen examined: **MEXICO, Hidalgo**, Mpio. de Tenango de Doria, Ejido Muridores a 2 km de Apulco vía Agua Blanca, 20°20′05″N 98°20′52″W, alt. 2200 m, on rotten wood, 3 IX 1980, Cifuentes 827 (FCME 10620).

Guepiniopsis buccina (Pers.) L.L. Kenn., Mycologia 50(6): 888. 1959. Fig. 3h

Specimen examined: **MEXICO, Hidalgo**, Mpio. de Calnali, a 8 km de Ahuacatlan, 20°49′N 99°03′W, alt. 1300 m, on rotten wood, 8 VII 1980, Cifuentes 480 (FCME 10159).

Results

Calocera macrospora and *C. viscosa* have uniformly thick walls. In *C. viscosa* the wall layers are not defined, but they are in septa, while in *C. macrospora* the layers are clearly defined. A septal pore-like structure was observed only in *C. macrospora*.

Dacrymyces dictyosporus has both transverse and longitudinal septa, while only longitudinal septa are present in *D. chrysospermus*. The spore walls of these two species are thick, with four well-differentiated layers, and 3-layered septa are visible in each one. The *D. dictyosporus* septa have a wall with a central swelling (pore-like structure) that is not observed in *D. chrysospermus*.

Spore walls are not uniformly thick within *Dacryopinax*. In *Da. elegans* spore walls are thick but with undefined layers; two septal wall layers can be seen, but no septal pores are distinguished. Spore walls in *Da. spathularia* are thick (but not to the same degree as in *Da. elegans*) and the walls are 3-layered. In *Da. lowyi* (Sierra & Cifuentes 2005), a hilar appendix, broken hilum wall, and septal pores with a dolipore-like structure can be discerned.

Spore wall layers are visible in *Guepiniopsis buccina* and there is a pore-like structure in the septum. In *G. alpina* there is a swollen part in the transverse septal wall that is not dolipore-like. The results are summarized in Table 1.

Discussion

All our conclusions are based on well-fixed material, but the material may have been damaged during drying before fixing, and we could not make serial sections. Nonetheless, the presence or absence of spore septal pores

Table 1. Presence or absence of spore wall layers and septa with dolipore.

Species	Lateral spore wall a distinct layer	Spore septa with dolipore
Calocera macrospora	+	+
C. viscosa	–	–
Dacrymyces chrysospermus	+	–
D. dictyosporus	+	+
Dacryopinax elegans	–	–
Da. lowyi	–	+
Da. spathularia	+	–
Guepiniopsis alpina	–	– (?)
G. buccina	+	+

and distinguishable lateral wall layers appear species specific in *Calocera*, *Dacrymyces*, *Dacryopinax*, and *Guepiniopsis*. The differences observed are unlikely to be based on fixation artifacts. We could not distinguish lateral wall layers in the well-preserved spores of either *Da. elegans* or *C. macrospora,* so that the absence of distinguishable lateral wall layers in the less well preserved spores of *C. viscosa* is, therefore, not likely to result from poor fixation. On the other hand, the septal swelling seen in *G. alpina* might well be an artifact that requires further study for verification. Pore-like structures that can be detected in even poorly fixed material can easily be overlooked in single sections. In *Basidiomycota*, the absence of septal pores has been indicated as an important ultrastructural marker at the family and ordinal levels for of smut fungi (e.g., *Microbotryaceae*, *Georgefischeriales, Ustilaginales;* Bauer et al. 1997). Keller (1986, 1992, 1997), Keller & Job (1992), and Garnica et al. (2007) have all studied spore walls, yet none of them have commented on pores in the spore septa.

The morphology and DNA analysis of taxa within the *Dacrymycetales* does not appear to be consistent (Shirouzu et al. 2012). Perhaps some of the ultrastructural differences described here might be useful for characterizing taxa in a new classification more congruent with the DNA analysis. The first step might be in producing good serial sections for a better morphological reconstruction of septal pores in spores.

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